RAMBOLL PBPK MODEL AND MODEL VALIDATION

Harvey Clewell, PhD, DABT, FATS

P. Robinan Gentry, PhD, DABT

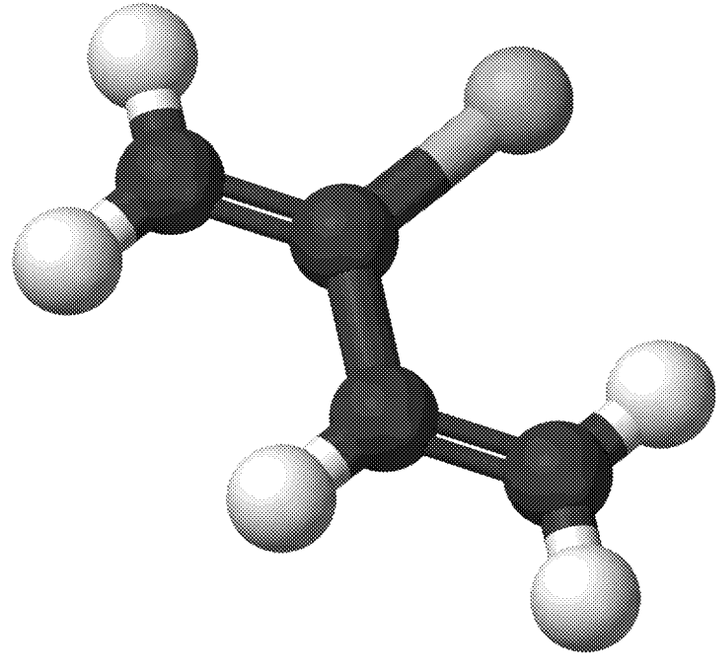
Jerry Campbell, PhD

Cynthia Van Landingham, MS

Melvin Andersen, PhD

Bruce Allen, MA

Sonja Sax, ScD



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OVERVIEW

- Background: History of interacting with USEPA on the chloroprene PBPK model as part of the Request for Correction
- Evidence demonstrating the need for a PBPK correction
- Why use a PBPK model?
- Mode of action considerations
- The updated PBPK model for chloroprene
- Model testing and validation
- Uncertainty analysis
- Conclusions

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HISTORY OF WORKING WITH USEPA ON CHLOROPRENE 2016-2020

2016

- Initial Denka Performance Elastomer (DPE)/Ramboll meeting with USEPA to discuss updating the 2010 IRIS Assessment, which included the Himmelstein (2004a,b) model.

2017

- USEPA conducted a quality review of an earlier published PBPK model (Yang et al. 2012) as part of the Request for Correction, raising concerns regarding its reliance on *in vitro* data.

2018

- Submitted DPE/Ramboll updated PBPK model to USEPA, and addressed questions raised during the Request for Correction review, including the reliance on *in vitro* data.
- Developed protocol for a USEPA-requested experiment to determine a chloroprene mass-transport parameter (Kgl).

2019

- Conducted the Kgl experiment with DPE based on an USEPA-approved protocol.
- Modified the Ramboll PBPK model to incorporate Kgl, considering discussions and recommendations from Dr. Schlosser.

2020

- January: Revised chloroprene PBPK model published in Inhalation Toxicology (Clewell et al. 2020).
- February: Chloroprene weight of evidence analysis published in print in Risk Analysis (Sax et al. 2020).
- April: Submitted chloroprene PBPK model documentation (Ramboll 2020) to USEPA for peer review.

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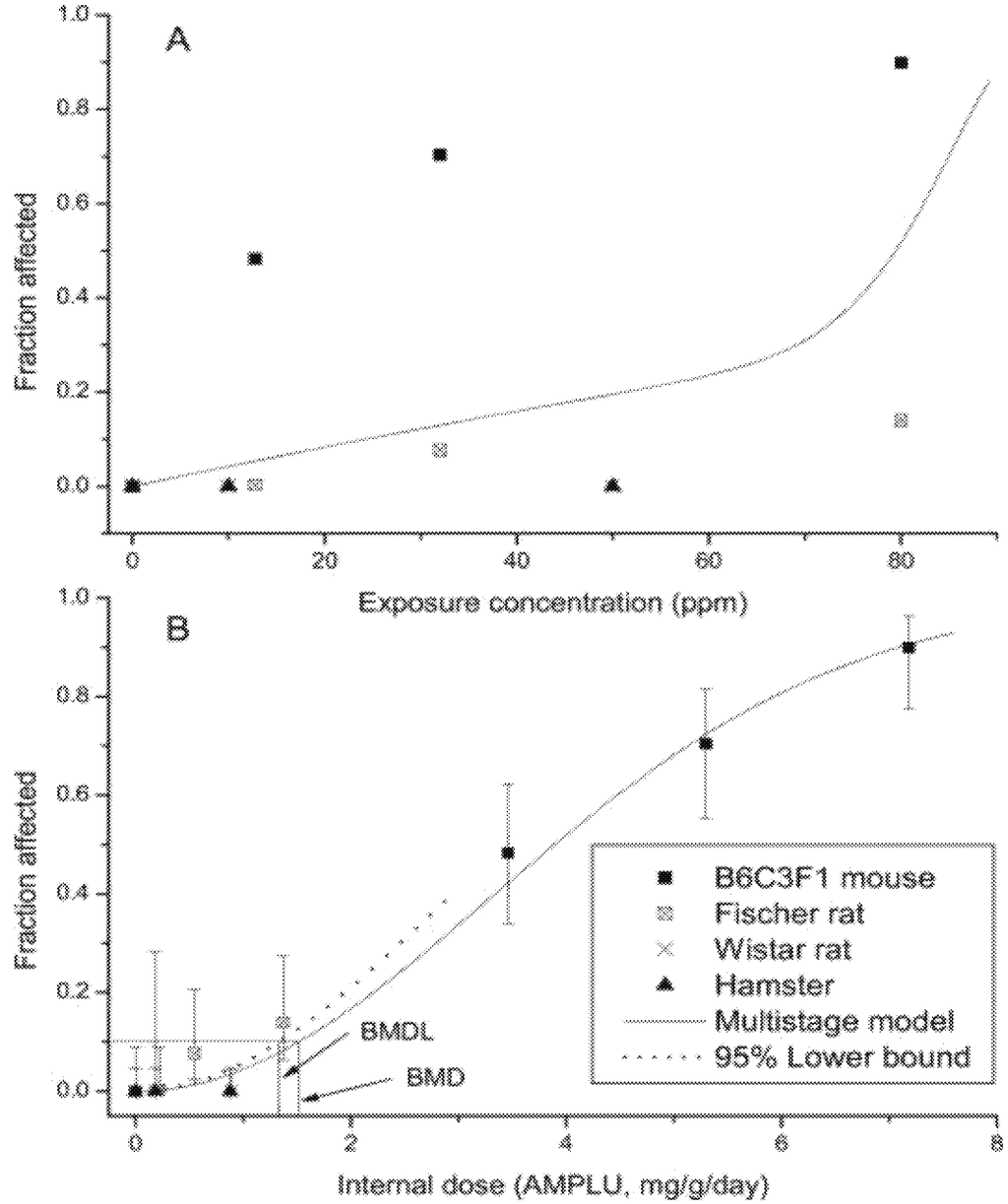
EVIDENCE DEMONSTRATING THE NEED FOR A PBPK CORRECTION

- Application of the PBPK model explains the differences across animal species (Himmelstein et al. 2004b), as well as the differences between animals and humans (Allen et al. 2014).
- USEPA (2010) IRIS Assessment for Chloroprene notes that "a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation."
- The USEPA (2005) "Guidelines for Carcinogen Risk Assessment" note that toxicokinetic or PBPK modeling is the preferred approach for estimating dose metrics from exposure.
- PBPK-derived estimates are necessary so that results are more consistent with cancer incidence observed in occupational studies; Marsh et al. (2007) found no evidence of excess cancer risk in a cohort of over 12,000 workers (1,100 from the Louisiana plant alone) i.e., none of the observed cancers were shown to be associated with chloroprene compared to local county cancer rates.
- Lung cancer incidence rates reported by the Louisiana Tumor Registry for St. John the Baptist Parish (where DPE is located) are lower than state cancer rates, indicating no excess lung cancers in the communities around the plant; other cancer rates are also lower or no different than state rates (Maniscalco et al. 2020).
- All the lines of evidence are outlined in Sax et al. (2020) and indicate a need for PBPK correction.

WHY USE A PBPK MODEL?

- Inhaled chloroprene concentration does not correlate with observed lung tumor incidence for different species in the chloroprene bioassays (top figure).
- The use of a PBPK model to predict total metabolism of chloroprene in the lung provided a consistent prediction of the lung tumor incidence in mice, rats and hamsters.
- The use of a dose metric based on tissue metabolism is consistent with the mode of action for chloroprene i.e., metabolic production of reactive epoxides.

Because mode of action matters!



Himmelstein et al. (2004b)

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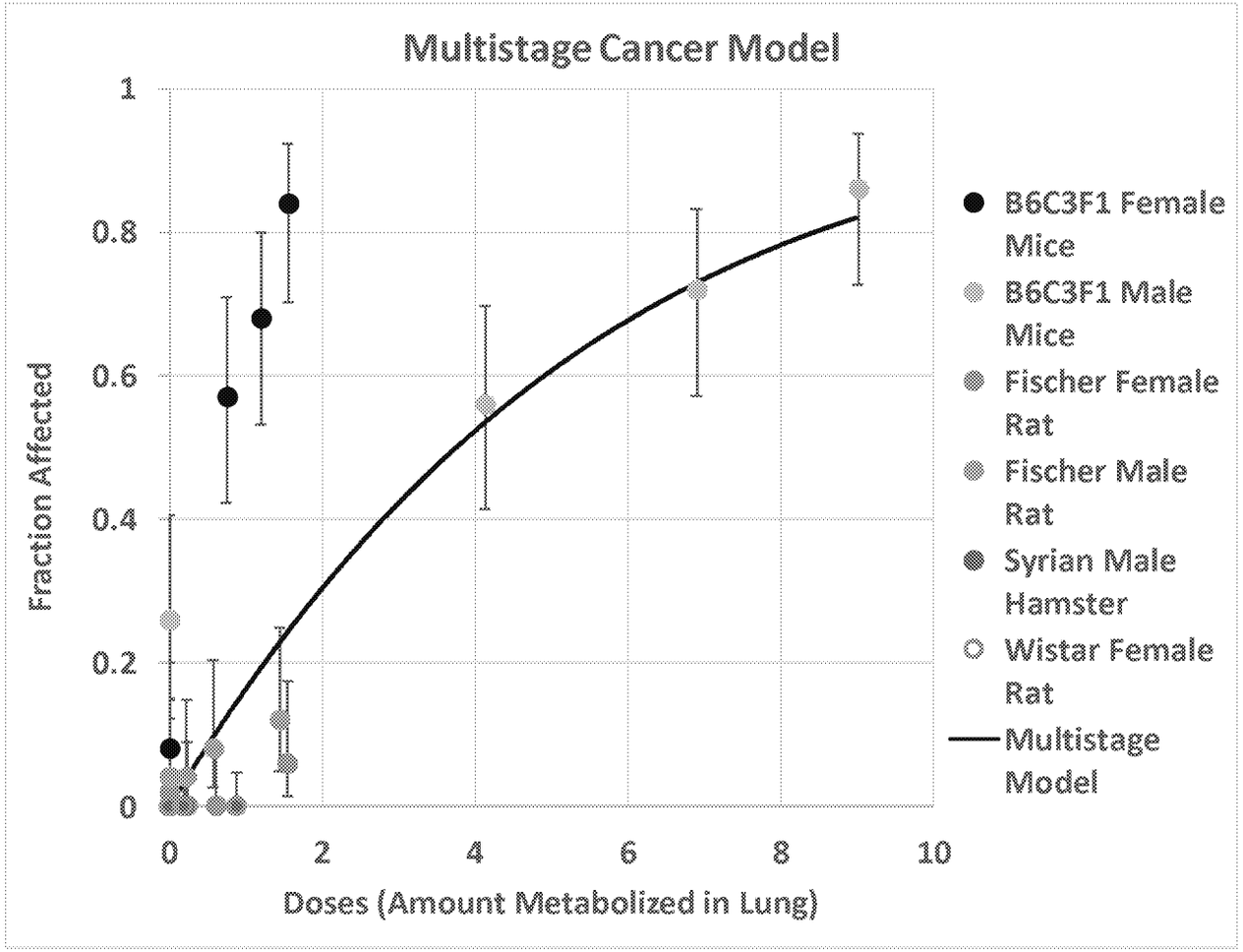
SENSITIVITY OF THE FEMALE MOUSE



Clewell et al. (2020)/Ramboll (2020)

- The revised PBPK model confirms the results from Himmelstein et al. 2004b, but indicates that, based on target tissue dose, the female mouse is more susceptible to the effects of the chloroprene epoxides compared to male mice and other species.
- The female mouse lung also demonstrated a more sensitive genomic response to oxidative stress from chloroprene than the female rat lung (Thomas et al. 2013).
- Studies with other chemicals provide evidence of a proliferative response to toxicity by Club cells in the female mouse lung that is not observed in the male mouse lung (Yamada et al. 2017) and is not explained by differences in metabolism (Van Winkle et al. 2002, Sutherland et al. 2012).
- Using the internal dose metrics from the highly susceptible female mouse results in a more conservative (higher) risk estimate.

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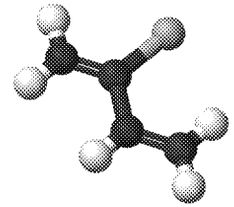
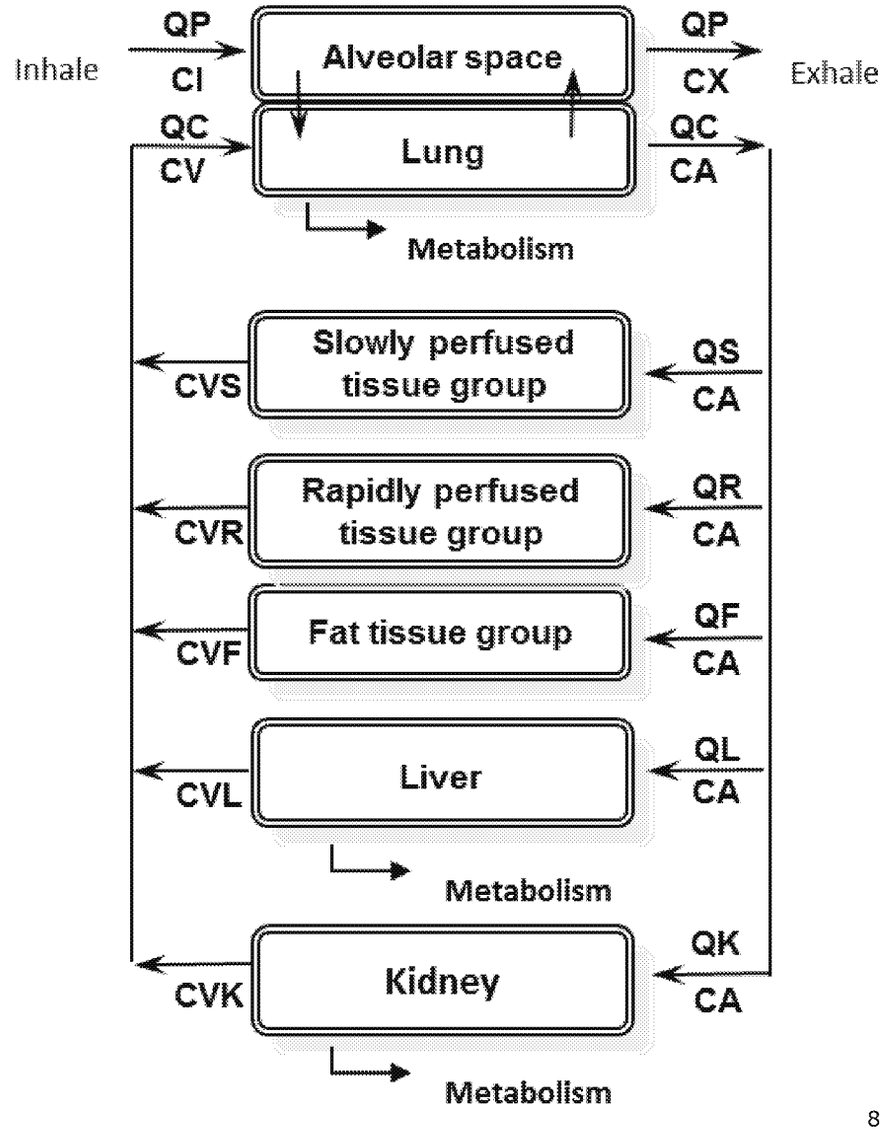
MODE OF ACTION CONSIDERATIONS



- Chloroprene is not in itself carcinogenic; tissue metabolism of chloroprene to highly reactive chloroalkyl epoxides is responsible for tumors observed in the cancer bioassays.
- The high reactivity of the chloro-epoxides limits their effects to the tissue in which they are generated.
- In contrast to the stable alkyl epoxides produced by the metabolism of chemicals like ethylene and butadiene, where clearance is by further metabolism and blood flow, the clearance of the chloroalkyl epoxides is by direct chemical reaction and is species invariant.
- Therefore, the appropriate dose metric is the total daily production of epoxides in the tissue of concern divided by the tissue volume (Andersen et al. 1987).
- Dose metrics based on chloroprene concentrations, whether in the inhaled air, blood or tissues, are not consistent with the mode of action and provide seriously erroneous estimates of risk for chemicals with the same mode of action as chloroprene (e.g., methylene chloride and vinyl chloride).

UPDATED CHLOROPRENE PBPK MODEL

- Structure based on PBPK model of methylene chloride (Andersen et al. 1987).
- Parameters obtained from the literature:
  - Physiological parameters: Brown et al. (1997)
  - Partition coefficients: Himmelstein et al. (2004b)
  - Metabolism parameters: Himmelstein et al. (2004a) and Yang et al. (2012)
- Code: R programming language
  - R-scripts for running mouse validation study and dose metrics in mouse, rat and human.
  - Documentation provided for all parameters.



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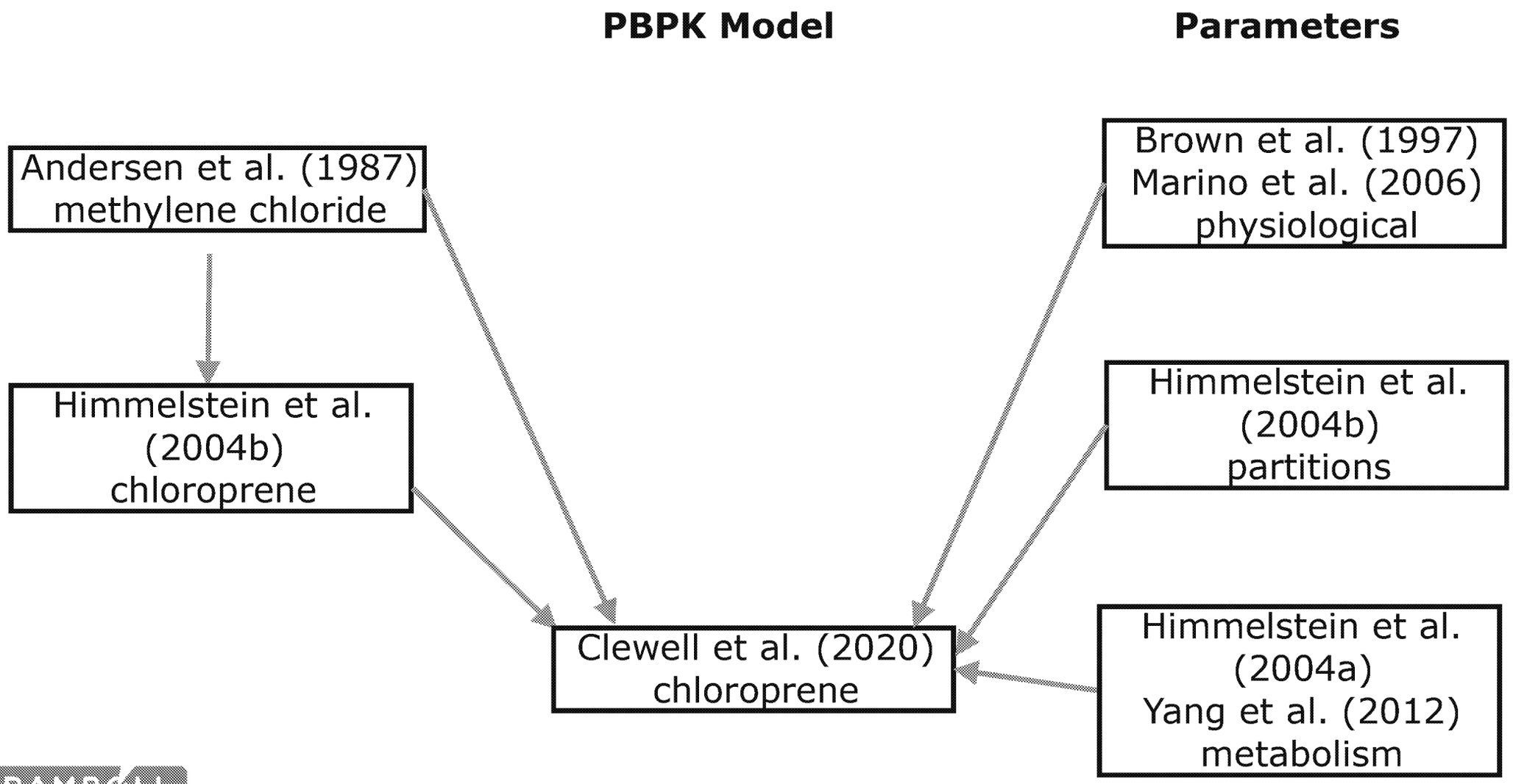
UPDATED CHLOROPRENE PBPK MODEL



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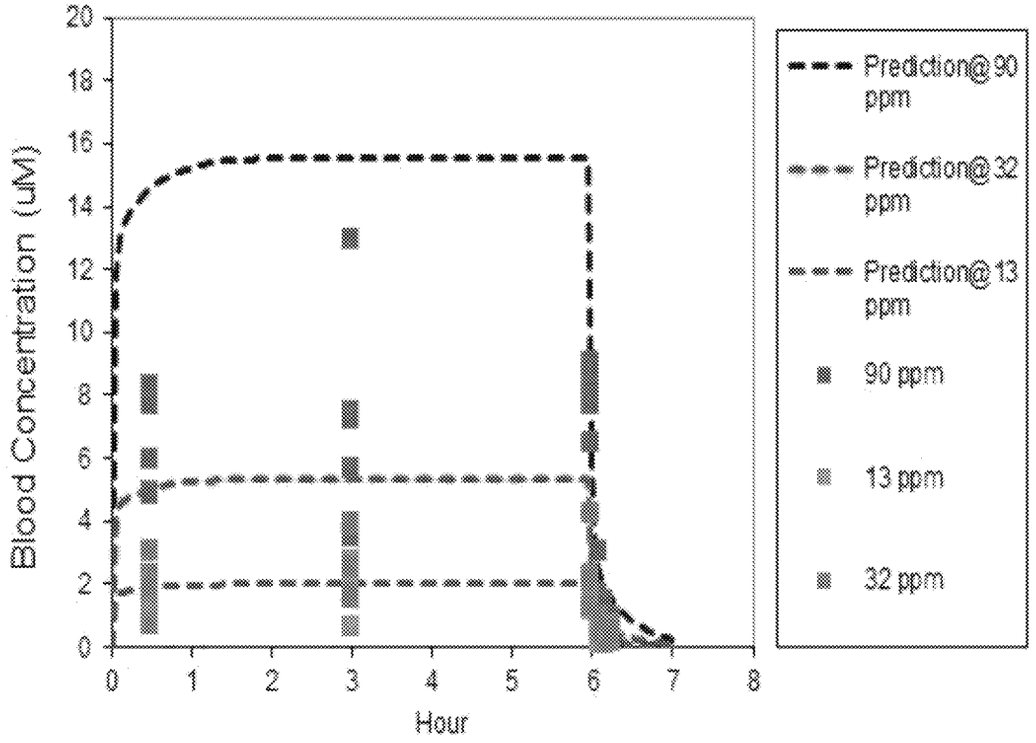
MODEL TESTING AND VALIDATION ANALYSES

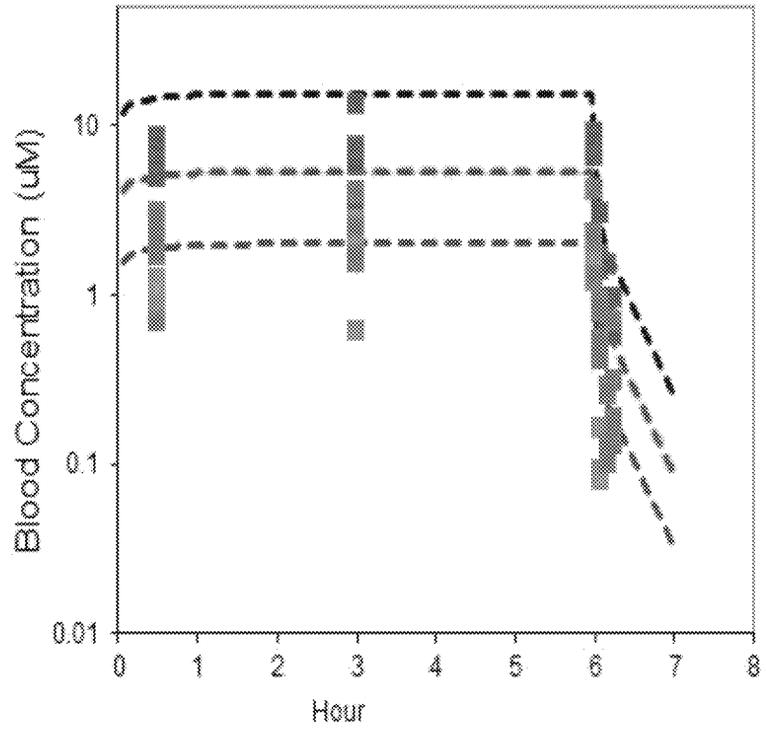
- Validation against the *in vivo* data
  - Ramboll tested the chloroprene PBPK model and found it was able to reproduce the blood concentrations reported in both the single and repeated exposure *in vivo* studies.
  - Ramboll evaluated the minute ventilation data from the chloroprene single exposure study and the metabolism induction data from the repeated exposure study and determined that there was no evidence of reduced ventilation or induction of metabolism in response to chloroprene exposure.
- Re-estimation of model parameters and consistency across tissues and genders
  - At the request of USEPA, Ramboll investigated the impact of re-estimating the published estimates from Yang et al. (2012) using an additional estimated mass transport parameter (Kgl) suggested by USEPA.
  - Ramboll conducted an analysis of the impact of the alternative parameter estimates on resulting dose metrics.
- Scale-up of in vitro data
  - A metabolism expert, Dr. Miyoung Yoon (now with USFDA), collaborated with Ramboll on the approach for conducting quantitative *in vitro* to *in vivo* extrapolation of the *in vitro* metabolism data.

VALIDATION OF THE MODEL

- 6-hour inhalation exposures of female mice to chloroprene (Clewell et al. 2020).
- The model predictions fit the in vivo results very well (within a factor of 2 of the means of animal data) with no adjustment of parameters.



Linear plot (concentrations)

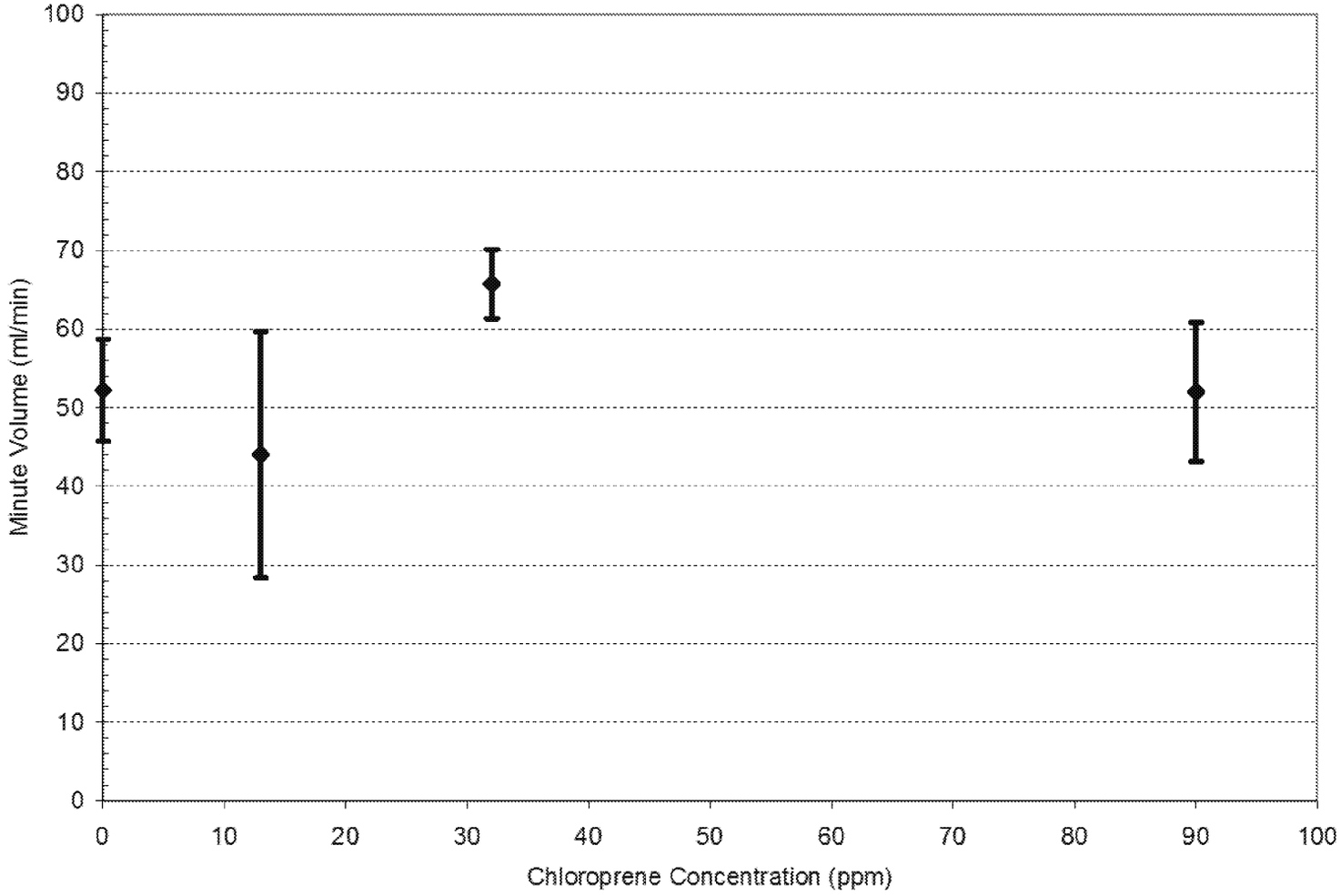


Log plot (rates)

VALIDATION OF THE MODEL

Minute ventilation during 6-hour inhalation exposures of female mice to chloroprene (Clewell et al. 2020)

- Plot shows measured pulmonary ventilation (ml/min) as a function of chloroprene concentration.
- Results show that minute volume is not associated with chloroprene concentrations.
- This suggests that respiratory depression was not an issue.
- Alveolar ventilation used in PBPK model corresponds to average measured value.



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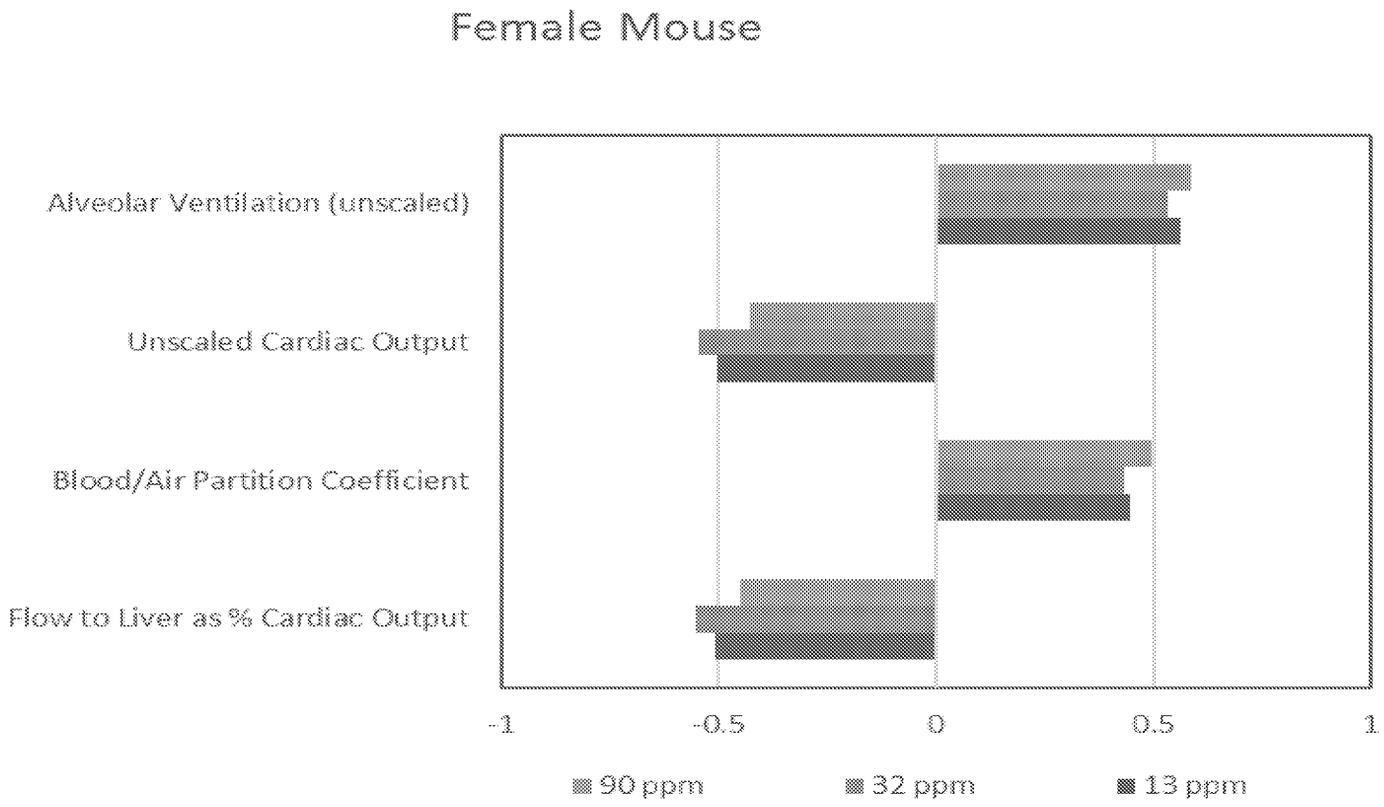
MODEL PARAMETERS: SENSITIVITY OF BLOOD CONCENTRATION (CVLC) TO CHANGES IN THE MODEL PARAMETERS



All sensitive parameters are either:

- directly measured (ventilation, blood/air partition) or
- obtained from physiological literature (cardiac output, liver blood flow)

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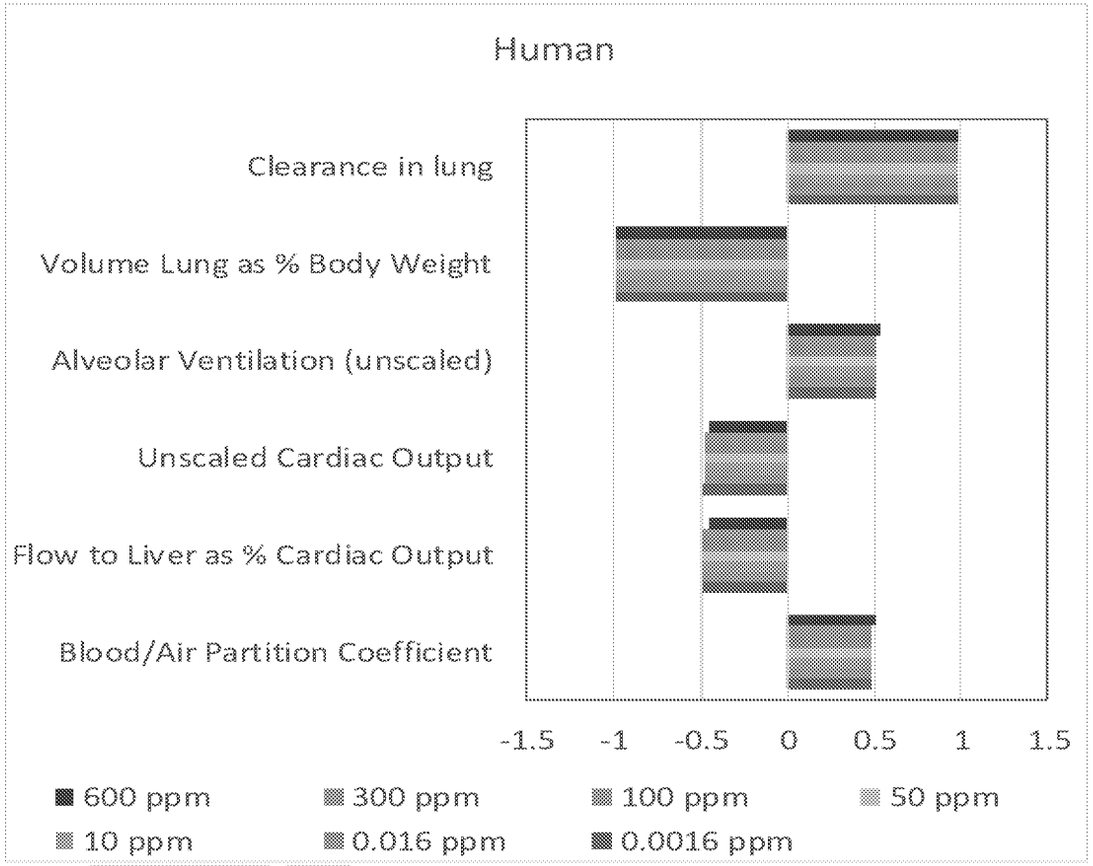
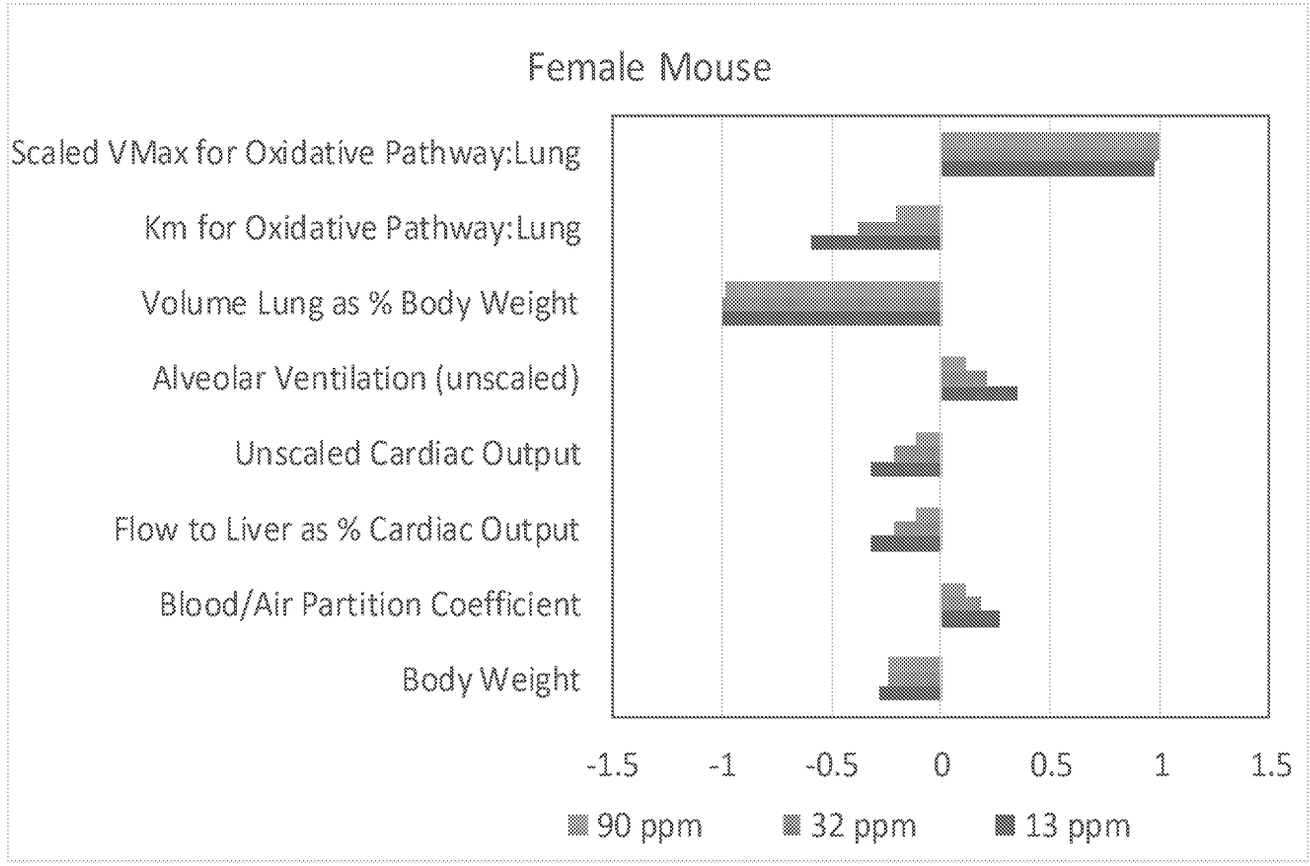
MODEL PARAMETERS: SENSITIVITY ANALYSIS OF AMOUNT METABOLIZED IN THE LUNG DAILY PER GRAM OF TISSUE (AMPLU) TO CHANGES IN THE MODEL PARAMETERS

As expected, the lung dose metric is sensitive to the same parameters as the in vivo study, plus lung metabolism and lung volume.





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INVESTIGATION OF TRANSPORT LIMITATION (KGL) DURING IN VITRO METABOLISM STUDIES

- USEPA raised questions regarding the transfer of chloroprene from the air to the media (Kgl) in the vials and how this could have affected the observed clearance rates reported in Himmelstein et al. (2004a) metabolism studies.

- At the request of USEPA, a new experimental study was performed to estimate a Kgl for chloroprene, following a protocol based on a benzene study conducted by Schlosser et al. (1993).

- The application of these data into the model demonstrated that the experimental value of Kgl obtained in this study was inconsistent with the high rates of liver metabolism reported in Himmelstein et al. (2004a).

- Therefore, Ramboll re-estimated the Kgl from the metabolism study data using an approach suggested by Dr. Schlosser (personal communication), which is based on the ratio of the mixing rates in the new Kgl study and the Himmelstein et al. (2004a) study.

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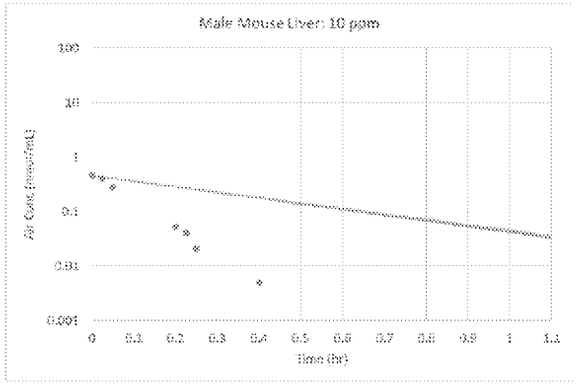
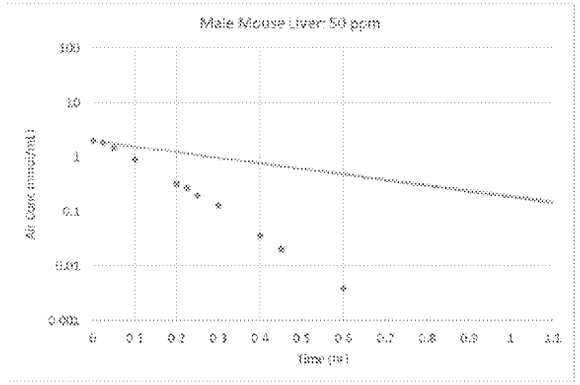
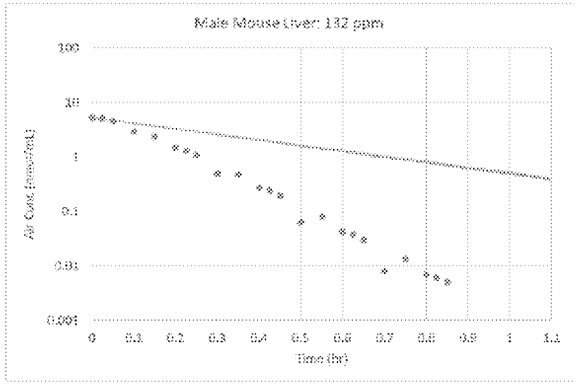
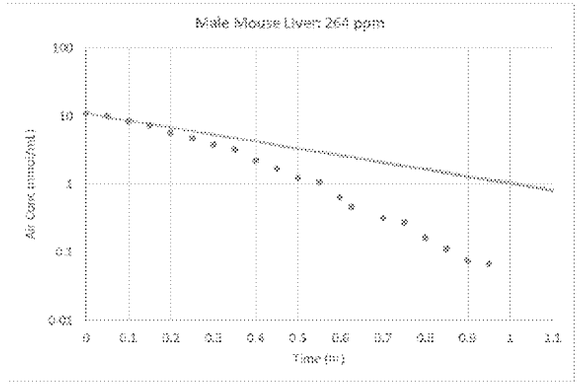
TRANSPORT LIMITATION (KGL) MCMC EVALUATION

Experimental Kgl = 0.020 L/hr
(95% CI. = 0.015 – 0.036)



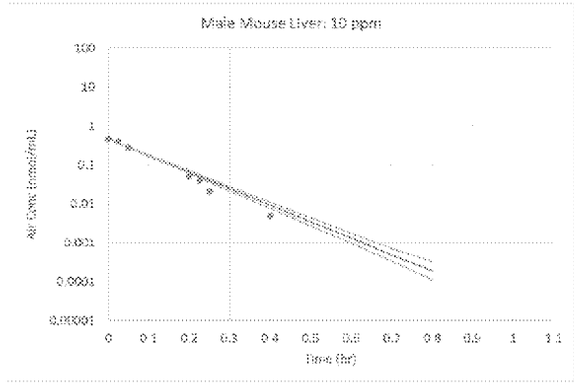


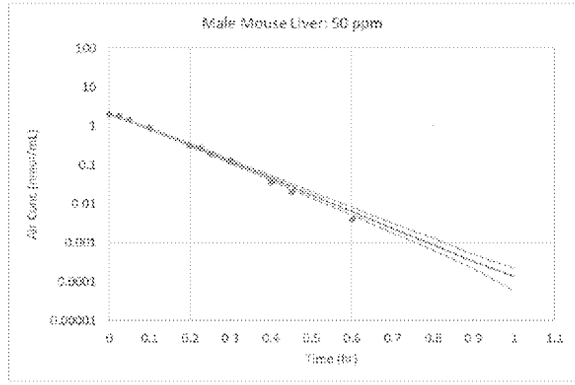


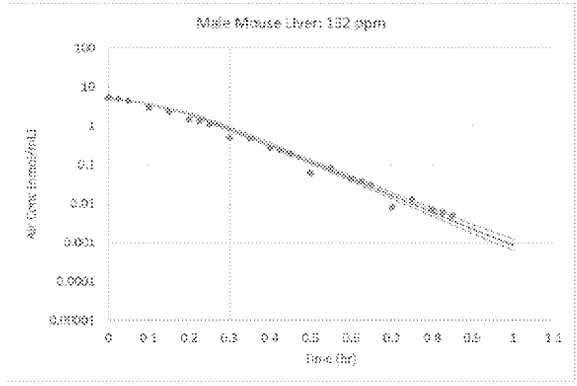


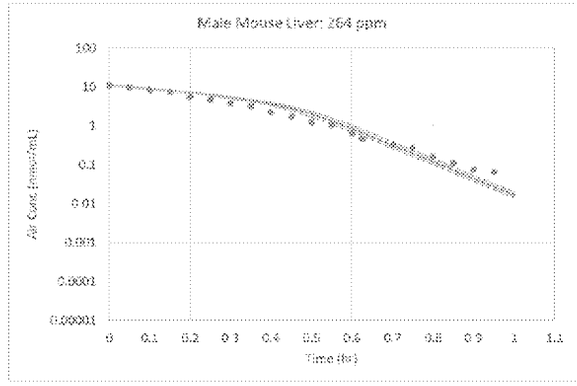
* Cannot fit metabolism data if Kgl < 0.11 L/hr

Estimated Kgl = 0.45 L/hr*
(95% CI. = 0.34 – 0.65)





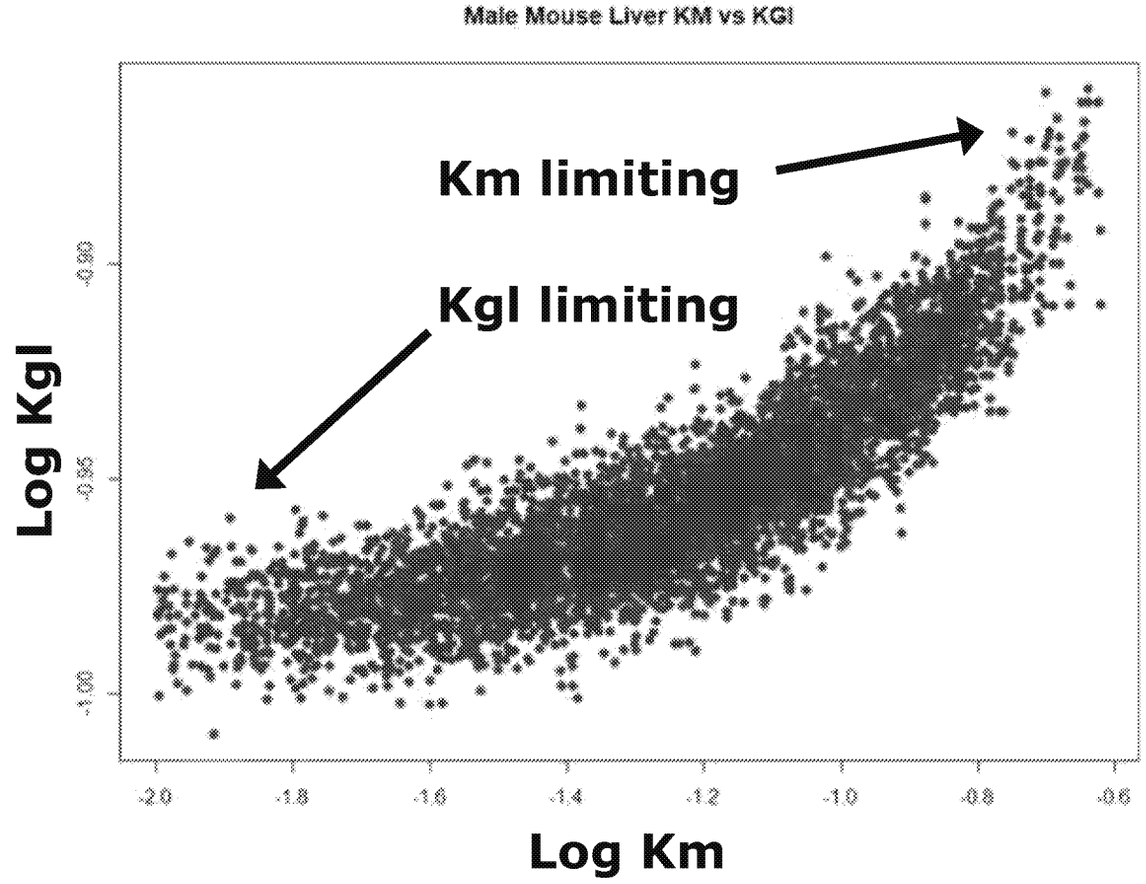




* Estimated from male mouse liver metabolism data, with Km = 1 μM

EFFECT OF ASSUMING A TRANSPORT LIMITATION (KGL)

- As Kgl decreases, it competes with metabolism, decreasing clearance of chloroprene in the vial.
- The effect of introducing Kgl into the metabolism parameter estimation is to reduce the estimated Kms in the tissues to implausible values, much lower than the range of 1-7 µM observed *in vivo* for other CYP2E1 substrates.



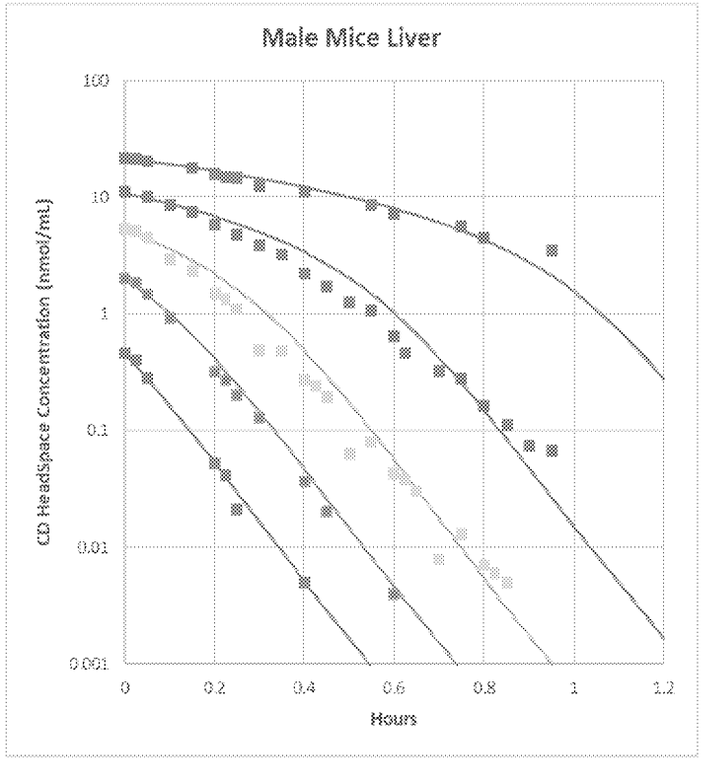
CONSIDERATIONS REGARDING KGL

- An experimental Kgl is critically dependent on the nature of mixing. It is difficult to apply a Kgl estimated from one experimental design to another, different design.
- As mixing increases, the transition from diffusion to laminar convection and then to turbulent convection increases the rate of mass transfer in a nonlinear manner.
- Based on the experimental metabolism data, we believe that more effective mixing and non-specific binding to microsomes increased the rate of transport of chloroprene in those studies.
- The investigator who conducted the study considered the possibility of slow mixing when he designed the study and is confident that the system was well mixed.
- We were able to obtain an acceptable fit to the data without using Kgl.
- Incorporating this additional, unsupported parameter (Kgl) results in a more uncertain analysis.

ALTERNATIVE APPROACHES FOR ESTIMATING METABOLISM

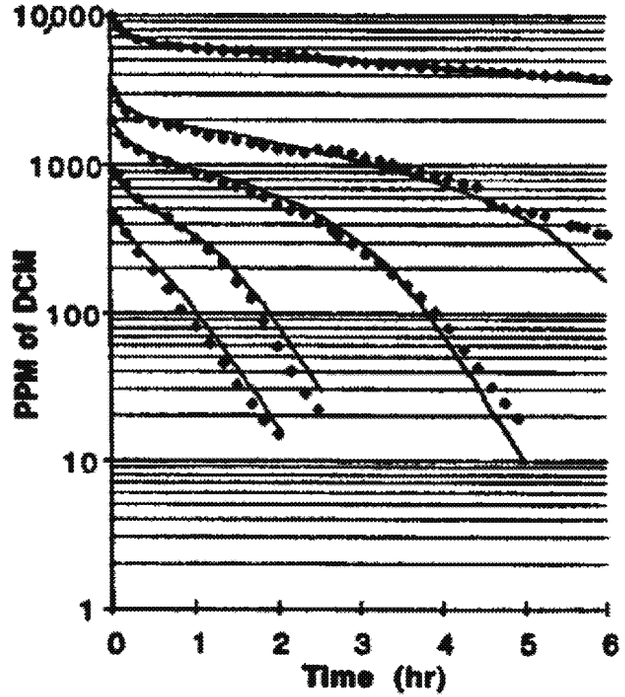
Chloroprene (2020)

In Vitro Data



Methylene Chloride (1987)

Closed Chamber Data

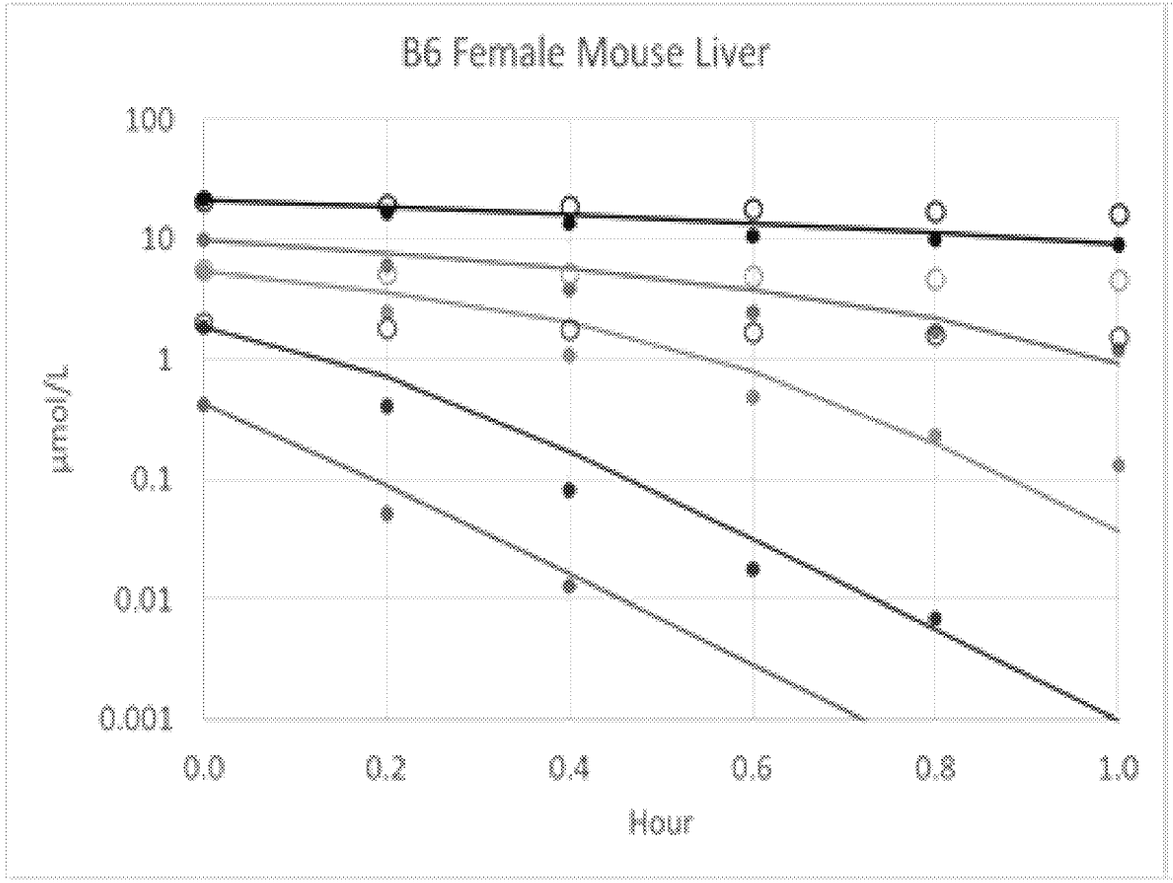


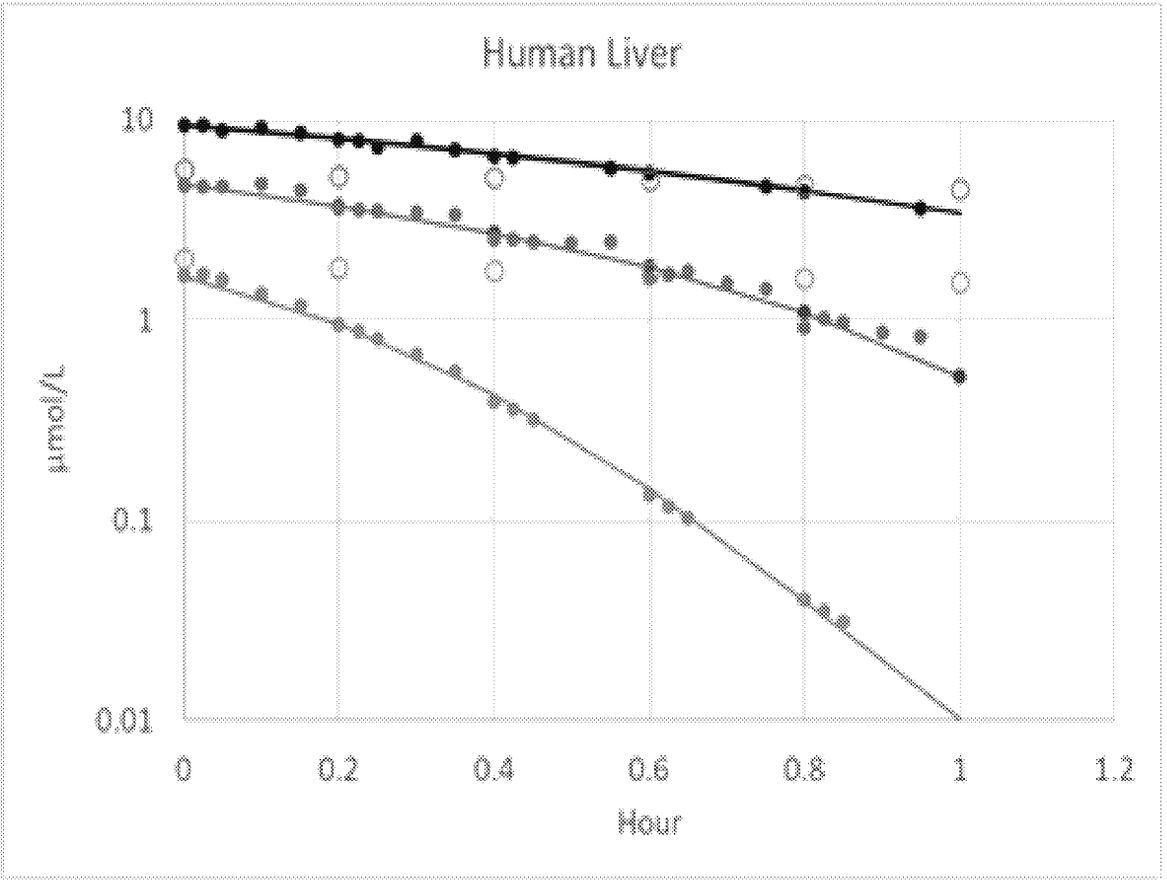
Andersen et al. (1987)

Both *in vitro* and *in vivo* data can be used to estimate metabolism

KINETIC ANALYSIS OF IN VITRO DATA: MOUSE AND HUMAN LIVER

Because the data spans concentrations from above to below saturation it was possible to estimate reliable values of both the capacity (Vmax) and affinity (Km) of metabolism.



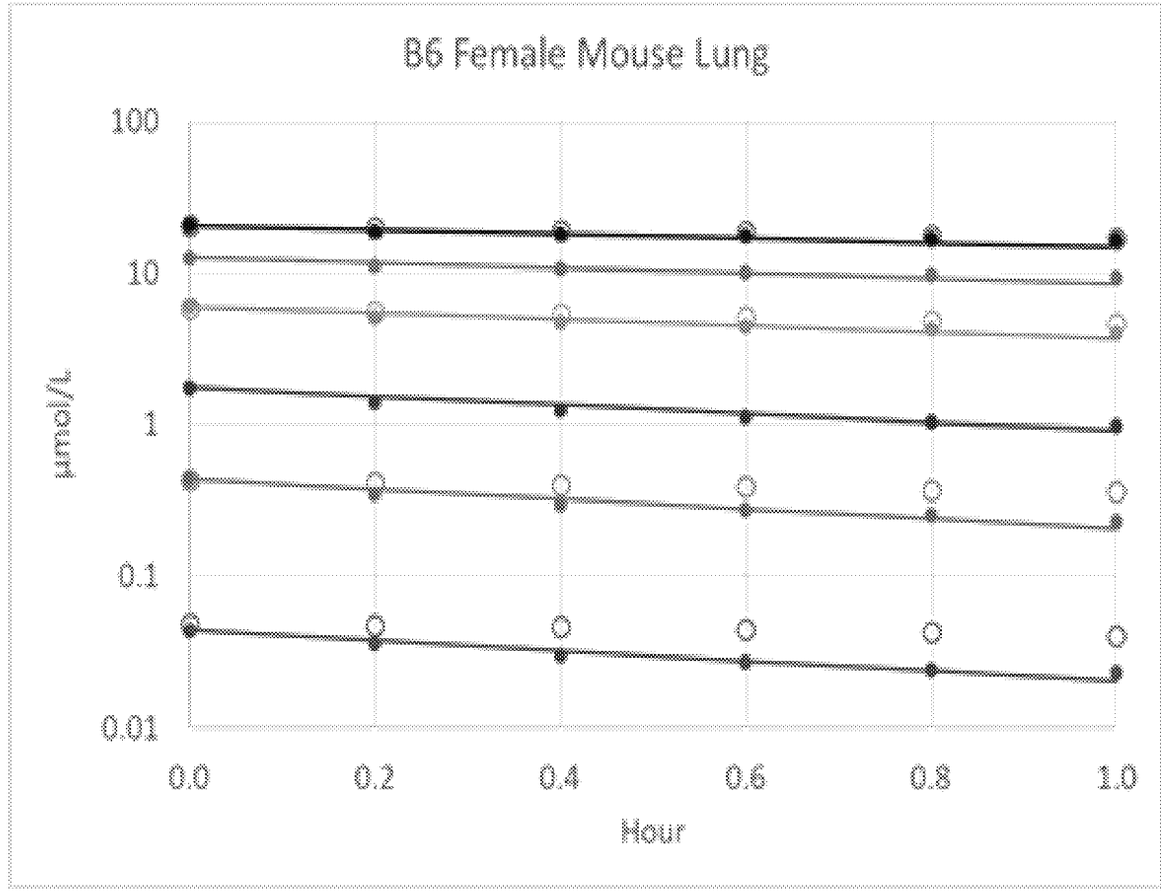


Open circles: control vials; Solid symbols: metabolism vials

Ramboll (2020)

KINETIC ANALYSIS OF IN VITRO DATA: MOUSE LUNG

Because the data spans concentrations from above to below saturation it was possible to estimate reliable values of both the capacity (Vmax) and affinity (Km) of metabolism.



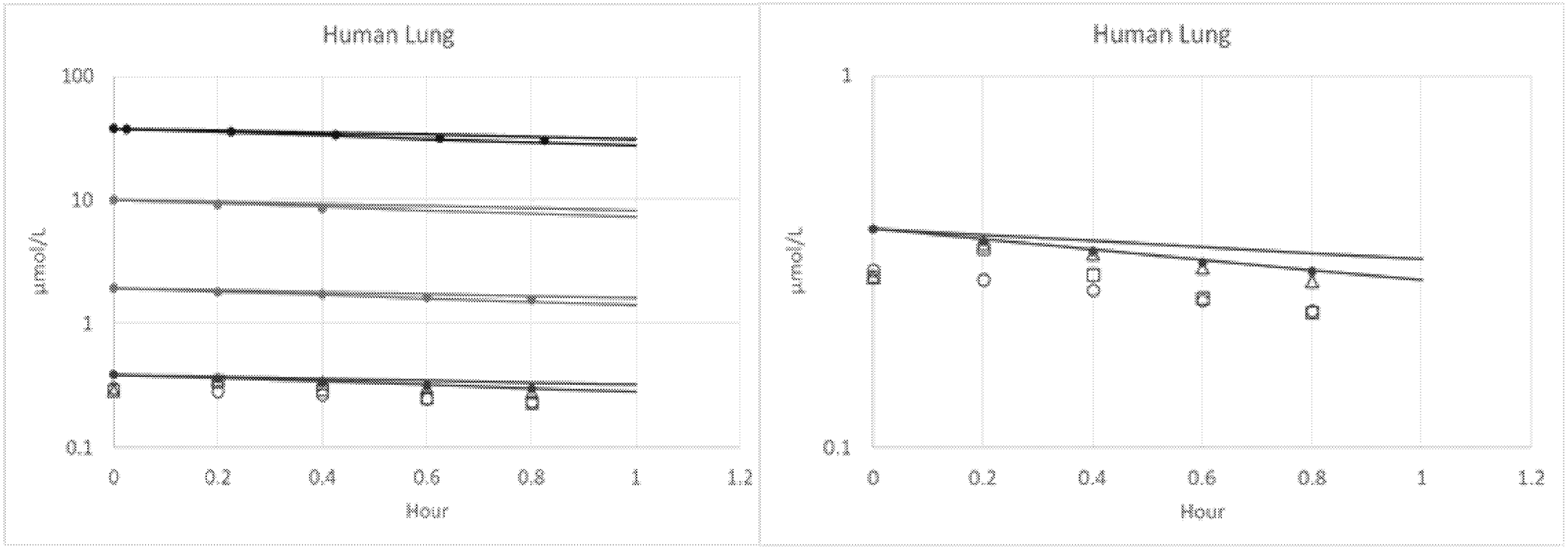
Ramboll (2020)

Open circles: control vials; Solid symbols: metabolism vials

KINETIC ANALYSIS OF IN VITRO DATA: HUMAN LUNG

Metabolism in the human lung is so slow that it was not possible to estimate reliable values of both the capacity (Vmax) and affinity (Km) of metabolism.

Rate of loss in metabolism vials is less than in controls.



Open symbols: control vials; Solid symbols: metabolism vials

Ramboll (2020)

The EPA proposed uncertainty analysis of the human lung metabolism is not needed and will not be reliable

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SCALE-UP OF IN VITRO METABOLISM DATA

- The approach used in this effort was designed by Dr. Miyoung Yoon (now with USFDA), an internationally recognized expert in IVIVE, and reflect the state of the art for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE).
- QIVIVE should not be confused with rapid screening IVIVE approaches such as the USEPA *httk* modeling software, which is designed to make rapid predictions with minimal data to support interpretation of HTS results.
- The USEPA Office of Pesticides has accepted the use of PBPK models using IVIVE of microsomal metabolism data to support their evaluations of early life sensitivity to pesticides.
- The FDA routinely accepts microsomal metabolism data and PBPK modeling to predict drug-drug interactions *in vivo.*
- Uncertainty in the human lung metabolism of chloroprene was addressed using the approach from the USEPA (2011) methylene chloride IRIS assessment, which used a measure of the relative CYP abundance in human liver and lung (Andersen et al. 1987).

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THERE IS UNCERTAINTY IN METABOLISM PARAMETERS DERIVED FROM *IN VIVO* STUDIES TOO

Development of a Physiologically Based Pharmacokinetic Model of Trichloroethylene and Its Metabolites for Use in Risk Assessment

CLEWELL ET AL. (2000)

Table 1. Parameter values used in the PBPK model for TCE.

Parameter	Abbreviation	Units	Mouse	Rat	Human
TCE metabolism					
Capacity	VMC	mg/hr[a]	39* (39.–60.)	12* (12.–20.)	10* (6.–10.)
Affinity	KM	mg/L	0.25	0.25* (0.25–18.)	1.5* (1.5–3.)
Fraction TCA	PO	–	0.035* (0.035–0.1)	0.02* (0.02–0.06)	0.08

Different metabolism parameters were required to fit each study.

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UNCERTAINTY ANALYSIS

- An uncertainty analysis was conducted on the PBPK model and the results are presented in the publication documenting the application of the model in a risk assessment for lung tumors (Clewell et al. 2020).
- The Ramboll (2020) report does not estimate quantitative uncertainty in the PBPK model because the USEPA specifically requested that the report not include any discussion related to estimation of risks.
    - It is our understanding that the USEPA intends to conduct additional uncertainty analyses to evaluate the impacts on the cancer unit risk estimate.
- For this review we performed a comparison of the dose metric predictions obtained with the newly revised chloroprene PBPK model against those obtained using the original published model (Yang et al. 2012).
    - Despite major differences in the approaches taken for metabolism parameter estimation and *in vitro* to *in vivo* extrapolation, the two model versions produce almost identical dose metrics, demonstrating the robustness of the PBPK model predictions.

COMPARISON OF DOSE METRIC PREDICTIONS

Exposure	Concentration	Ramboll 2020 Dose Metric*	Yang et al. 2012 Dose Metric*
Female Mouse Bioassay	12.8 ppm	1.00	0.75
	32 ppm	1.58	1.2
	80 ppm	2.15	1.57
Human Continuous Exposure	1 μg/m^3	3.36x10^{-6}	2.7x10^{-6}

* average mg metabolized per gram lung per day

CONCLUSIONS

- PBPK modeling is the preferred approach for cross-species extrapolation (USEPA 2005) because it considers the large pharmacokinetic differences demonstrated between mice and humans for chemicals such as chloroprene.
- A validated PBPK model has been developed and documented, and the results have been published in a peer-reviewed journal (Clewell et al. 2020).
- The Ramboll team appreciates the interaction with USEPA scientists to further validate and improve the model and make it accessible to others.
- We have high confidence in the chloroprene PBPK model due to its similarity to previously accepted PBPK models and the robustness of the *in vitro* data on which it is based. It is likely better than data you would obtain from in vivo studies. Kenyon et al. (2020) have shown that *in vitro* estimates of metabolism for similar volatile organic compounds are generally within a factor of two to three of estimates inferred from *in vivo* studies
- Ramboll has determined that the impact of uncertainties in the PBPK model is small compared to the impact associated with ignoring important species differences in target tissue dosimetry (i.e. relying on default assumptions).
- The PBPK model indicates a need for revising the 2010 IRIS assessment to provide a corrected cancer unit risk based on the best available science.

THANK YOU

Robinan Gentry
rgentry@ramboll.com
Harvey Clewell
hclewell@ramboll.com
Mel Andersen
andersenme@aol.com
Bruce Allen
Bruce.C.Allen@outlook.com
Cynthia Van Landingham
cvanlandingham@ramboll.com
Jerry Campbell
jcampbell@ramboll.com
Sonja Sax
ssax@ramboll.com

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